Adult Reproductive Capacity of *Ceratitis capitata* (Diptera: Tephritidae) on a Chemically Defined Diet

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ABSTRACT A chemically defined diet (*Ceratitis capitata* #2 diet) for rearing adult *Ceratitis capitata* (Wiedemann), the Mediterranean fruit fly, was developed by adding vitamin B_{12} 0.024 mg, ascorbic acid phosphate (vitamin C) 3.68 mg, vitamin D 12 mg, vitamin E 8.16 mg, inositol 243 mg, choline chloride 120 mg, cholesterol 40 mg, nicotinic acid 1.64 mg, sugar 6 g, agar 400 mg, and water 7 ml to each 50 g meridic larval diet (*C. capitata* #1). The *C. capitata* #2 diet was prepared in two forms, agar and liquid and both showed comparable performance to the standard diet but the agar diet is considered to be the best diet tested. Adults reared on the agar diet produced more eggs than those on a protein hydrolysate-sugar (1:3) diet. Flies fed on diets lacking nutrient groups, such as the 10 essential amino acids, eight nonessential amino acids or the combination of cholesterol, inositol, and choline, produced fewer eggs. Those fed on the diet without the 14 vitamins, cholesterol, inositol, or choline produced a normal number of eggs. Increasing the sugar content in the diet did not affect egg production or hatch. Sugar concentration in the diet influenced fly survival.

KEY WORDS *Ceratitis capitata*, diet, fruit fly, defined diet, reproductive capacity, development

INSECT DIETS AFFECT the performance of insects during immature and adult stages. Adult emergence, female size, preoviposition egg production and larval development period are affected during the immature stage, whereas effects on postoviposition egg production, diet ingestion, sexual acceptance, or longevity (survival) occur during adulthood (Cangussu and Zucoloto 1997).

Adult nutrition studies have been conducted on tephritids throughout the last six decades. Gupta (1977) developed a chemically defined diet for *Dacus cucurbitae* (Coquillett) adults. Tsiropoulos (1980a, 1980b, 1985) identified the major nutritional requirements of adult *Bactrocera (=Dacus) oleae* (Gmelin) and emphasized the importance of vitamins in survival, egg production, and hatchability. Braga and Zucoloto (1981) investigated amino acid concentrations for adult *Anastrepha oblique* L. Tsiropoulos (1983) studied the importance of dietary amino acids on reproduction and longevity of adult *B. oleae.* Ferro and Zucoloto (1990) showed that sucrose did not affect egg production of *Ceratitis capitata* (Wiedemann) but amino acid composition of the diet did. Zucoloto (1992) studied egg production in *C. capitata* fed different carbohydrates. Kaur and Srivastava (1991, 1995) evaluated the effect of an artificial diet on various parameters of reproductive potential and the effect of host fruit on the preoviposition period of *D. cucurbitae.* Joachim-Bravo and Zucoloto (1997, 1998) reported that sucrose and citric acid in the diet influenced oviposition preference in *C. capitata.* Zografou et al. (1998) stated that amino acid analogues affect survival, fecundity, and fertility of *B. oleae.* Chang and Kurashima (1999) concluded that green bell pepper and ascorbic acid phosphate increase pupal recovery of *B. latifrons* (Hendel). Chang et al. (2000) developed a meridic larval diet (*C. capitata* #1) for *C. capitata.* We report here how a chemically defined diet (*C. capitata* #2) was developed and used to study the preliminary nutritional requirements for *C. capitata* adults.

Materials and Methods

Insects. *Ceratitis capitata* pupae were obtained from colonies of the "Maui med 93" strain maintained at U.S. Pacific Basin Agricultural Research Center, USDA–ARS, Honolulu, HI, for ≈60 generations. Newly emerged adults were reared on the standard protein hydrolysate:sugar (1:3) diet and the experimental chemically defined diet.

Diet Preparations. The composition of the *C. capitata* #2 diet is shown in Table 1. All chemicals listed in Table 1 were purchased from ICN Pharmaceuticals (Costa Mesa, CA). They were weighed, mixed, and prepared in a 100-ml beaker over a hot plate (55°C) with a stirrer (Daigger, Vernon Hills, IL) to form a liquid mixture. Agar was added to the mixture and cooked until the solution became clear. The agar mixture was poured into petri dishes and left to harden. Agar and liquid diets were stored in the refrigerator

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Table 1. Composition (mg/50 g diet) of *C. capitata* #1 larval and #2 adult diet

Constituents	#1[a]	#2 Agar	#2 Liquid
Essential amino acids	636.0	636.0	636.0
L-arginine	106.8	106.8	106.8
L-histidine	45.6	45.6	45.6
L-isoleucine	56.4	56.4	56.4
L-leucine	108.0	108.0	108.0
L-lysine	58.8	58.8	58.8
L-methionine	27.6	27.6	27.6
L-phenylalanine	70.8	70.8	70.8
L-threonine	54.0	54.0	54.0
L-tryptophan	28.8	28.8	28.8
L-valine	79.2	79.2	79.2
Non-essential amino acids	964.8	964.8	964.8
L-alanine	78.0	78.0	78.0
L-aspartic acid	112.8	112.8	112.8
L-cysteine	40.8	40.8	40.8
L-glutamic acid	392.4	392.4	392.4
L-glycine	90.0	90.0	90.0
L-proline	124.8	124.8	124.8
L-serine	78.0	78.0	78.0
L-tyrosine	48.0	48.0	48.0
Vitamins	36.35	425.85	425.85
Thiamin (Vitamin B_1)	1.00	1.00	1.00
Riboflavin (Vitamin B_2)	1.00	1.00	1.00
Nicotinic acid	1.00	2.64	2.64
Pantothenic acid (Coenzyme A)	1.00	1.00	1.00
Pyridoxine (Vit. B_6)	1.00	1.00	1.00
Folic acid	0.25	0.25	0.25
Biotin	0.10	0.10	0.10
Inositol	10.00	253.00	253.00
Choline chloride	20.00	140.00	140.00
ρ-amino benzoic acid	1.00	1.00	1.00
Ascorbic acid phosphate (Vitamin C)	—	3.68	3.68
Vitamin D	—	12.00	12.00
Vitamin E	—	8.16	8.16
Vitamin B_{12}	—	0.02	0.02
Ribonucleic acid	100.00	100.00	100.00
Sugar	2,000.00	8,000.00	8,000.00
Cholesterol	40.00	80.00	80.00
McCollum & Davis Salt mixture No. 185	100.00	100.00	100.00
Streptomycin	50.00	50.00	50.00
Oxytetracycline HCL	5.00	5.00	5.00
Methylparaben	100.00	100.00	100.00
Sodium benzoate	100.00	100.00	100.00
Citric acid	500.00	500.00	500.00
Corncob 30/80	12,000.00	—	—
Agar	—	400.00	—
Distilled water	33,000.00	40,000.00	40,000.00

[a] In Chang et al (2000).

(4°C) for later use. The protein hydrolysate:sugar diet (1:3) was used as the standard diet.

Diet Development. The adult chemically defined *C. capitata* #2 diet is a modification of the larval diet *C. capitata* #1. The additions were agar 400 mg, vitamin B_{12} 0.024 mg, ascorbic acid phosphate 3.68 mg, vitamin D 12 mg, vitamin E 8.16 mg, nicotinic acid 1.64 mg, inositol 243 mg, choline chloride 120 mg, cholesterol 40 mg and sugar 6 g. Corncob was removed from the *C. capitata* #1 diet and water content was increased (Table 1).

Bioassays. Nine grams of pupae (≈1,000 pupae) were set up for each treatment and flies allowed to emerge in a metal screen cage (24 by 24.5 by 27.5 cm). Test diets were placed inside cages as flies emerged. A water source was provided and fresh diets were furnished every other day. The flies fed ad libitum.

Evaluation Criteria. The efficacy of the diets was determined by evaluating fecundity, fertility, survivorship, mating ability, and flight ability as compared with flies on the standard diet.

At 5 d posteclosion, 10 mating pairs of *C. capitata* from each treatment were transferred to a 1-liter waxed cup with test diet provided in a vial cap (1 cm diameter) at the bottom. Eggs from test diet-fed females were collected and counted every other d starting from 6 d postemergence for 18 d (nine collections) from both egging devices and food. The egging device was made by inserting a bottomless sauce cup into another with a piece of 25-pinholed paraffin between. The number of eggs per female per day (fecundity) was calculated as the 18 d cumulative eggs counted divided by the mean number of females surviving and the number of collecting days. Four collections from each test diet were performed. Dead flies from each waxed cup were recorded by sex every other day and discarded. Percent survivorship was calculated as total flies (10 each sex) minus dead flies divided by 10 times 100. Four hundred eggs collected each day from 6-, 7-, 8-, or 9-d-old females fed on test diets were used to determine fertility (egg hatch).

Newly emerged *C. capitata* adults were sexed and placed in metal cages (24 by 24.5 by 27.5 cm) in a separate room with identical environmental conditions (75°F, 65%RH, and a photoperiod of 12:12 [L:D]) and supplied with test diets. To test flight ability, 25 flies of each sex, from each diet, were collected daily (on days 1–6) and placed in 1-liter wax cups. Flies were fast chilled in a −10°C freezer. As soon as they were immobile (≈1 min), the flies were removed from the freezer and flight ability was tested as described by Chang and Kurashima (1999). After 14 d, flies remaining in the dishes were counted as nonfliers and the numbers were expressed as a percent of the total. Percent flier was derived from 100% minus percent nonfliers. For the mating test, 25 virgin flies of each sex from each diet were introduced into an acrylic-mating cage (30 by 30 by 40 cm) at ages 3, 6, and 7 d old. Mating pairs were collected and counted every 5 min for a total of 60 min between 0800 and 1000 hours. Mating pairs were removed from the cages and recorded.

Nutritional Requirements. Grouped nutrients of 10 essential amino acids, eight nonessential amino acids, 14 vitamins or cholesterol, choline, and inositol as a whole were deleted from the chemically defined (*C. capitata* #2) diet to determine the importance of these groups of nutrients for adult *C. capitata.* The protein hydrolysate:sugar diet was again the standard diet.

Statistical Analysis. Data are reported as means ± SE. Differences among means with each diet were determined by analysis of variance (ANOVA), with the honestly significant difference (HSD) value calculated as a Tukey statistic at $\alpha = 0.05$ (SAS Institute 1996).

Table 2. Effect of presence or absence of nutrients on fecundity, fertility, and survival of adult *C. capitata*

Deleted items	Mean eggs/female/day		% survival		Egg hatch, %
	Exclude eggs on food	Include eggs on food	Females	Males	
Protein hydrolysate:sugar 1:3 (Standard)	11.88 ± 2.36ab	11.88 ± 2.36b	97.50 ± 0.70a	95.60 ± 1.20a	91.31 ± 0.75ab
None (*C. capitata* #2 agar diet, 17.6% sugar)	8.33 ± 2.34abc	22.23 ± 1.12a	85.60 ± 2.90ab	99.70 ± 0.30a	93.31 ± 0.92ab
10 Essential AA	4.96 ± 1.95abc	12.16 ± 1.10b	86.40 ± 2.30ab	100.00 ± 0.00a	90.06 ± 1.18bc
8 Nonessential AA	3.16 ± 0.70bc	13.56 ± 0.48b	93.90 ± 1.70a	98.10 ± 0.70a	89.88 ± 0.82bc
14 Vitamins	9.48 ± 2.94ab	20.18 ± 1.33a	88.90 ± 2.20ab	98.30 ± 0.60a	90.44 ± 0.98b
Cholesterol + Inositol + Choline	8.21 ± 0.96abc	13.05 ± 1.42b	81.10 ± 2.90b	100.00 ± 0.00a	94.94 ± 0.64a
6 g Sugar (*C. capitata* #2 diet, 4.4% sugar)	3.78 ± 0.54abc	16.98 ± 1.14ab	76.40 ± 4.70bc	82.20 ± 3.00b	86.44 ± 0.87bc
All except sugar	0.87 ± 0.35c	0.87 ± 0.35c	64.40 ± 4.10c	82.20 ± 3.40b	91.63 ± 0.66ab

Means within a column followed by different letters were statistically different ($\alpha = 0.005$, ANOVA test).

Results and Discussion

Diet Development. Before the *C. capitata* #2 adult diet was developed, our research focused on the combination of all analyzed nutrients (in chemical form) that were contained in protein hydrolysate and sugar (sucrose) to form a dry powder diet. Several unsuccessful attempts at diet development suggested that dry nutrients are not distributed or used evenly by flies. Furthermore, the idea that adult and larval flies may require similar nutrients for survival prompted us to rear adult *C. capitata* on an agar diet containing nutrients from the *C. capitata* #1 larval diet, supplemented with vitamins C, D, E, and B12, which are not present in the larval diet, as well as increased amounts of sugar, inositol, choline chloride, cholesterol, agar and water and elimination of corncob. Adults survived on this modified *C. capitata* #1 agar diet (subsequently *C. capitata* #2 diet) and fecundity was improved in comparison to the standard diet (protein hydrolysate: sugar 1:3). Flies reared on *C. capitata* #2 diet compared well with standard diet-fed flies with regard to survival, fecundity, fertility, and mating ability (Table 2; Fig. 1). Deletion of niacin, inositol, choline chloride, or cholesterol from the *C. capitata* #2 diet did not show any negative effects on fecundity and survival. We conclude, based on these results, that both larva and adult *C. capitata* require similar nutrients. Further precise studies will be needed to confirm the optimal amount of nutrients for adults or larvae.

Effect of *C. capitata* #2 Diet on Adult Development. *Fecundity.* The *C. capitata* #2 diet-fed flies produced significantly more total eggs than those fed on standard diet (Table 2). However, eggs oviposited from #2 diet-fed females into the egging device (excluding eggs on food) were not significantly different from those fed the standard diet. These results suggest that (1) the agar diet probably is a better oviposition site than the egging device and (2) nutrients in the defined diet apparently are more attractive for oviposition than water alone inside the egging device. This information may support the finding by Joachim-Bravo and Zucoloto (1997) that citric acid may be responsible for oviposition-site preference, because *C. capitata* #2 diet contains citric acid.

Survivorship. Survival of female and male flies fed *C. capitata* #2 diet was not significantly different from those fed the standard diet (Table 2). However, mortality of males was significantly increased (from 0.3 to 17.8%) with a decrease of the sugar concentration (from 17.6 to 4.4%) (Table 2).

Fertility. Egg hatch from *C. capitata* #2 diet-fed females was not affected in comparison with those fed the standard diet (Table 2).

Flight Ability. Flight ability measured 2–6 d post-adult eclosion did not differ among flies fed defined and standard diets. The data suggested there are sufficient nutrients provided in the *C. capitata* #2 diet for flight-muscle energetics.

Mating Ability. Mating frequency of flies, at age of 3, 6, or 7 d postadult emergence, from the *C. capitata* #2 diet-fed group was comparable to that of standard diet-fed flies. Mean data from these 3 d are shown in Fig. 1.

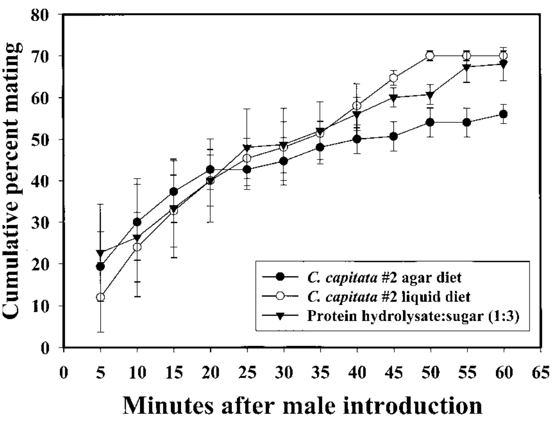

Fig. 1. Percent mating of flies maintained in three diets (protein hydrolysate:sugar 1:3; *C. capitata* #2 agar or *C. capitata* #2 liquid) fed to adult *C. capitata* at age 3, 6, and 7 d old. Mean data from these 3 d are shown. Reversed triangle: protein hydrolysate:sugar (1:3) diet. Solid circle: defined agar diet. Open circle: defined liquid diet. No significant differences were noted among the diets for 40 min after male introduction.

Table 3. Comparison of *C. capitata* #2 agar and liquid diets to the standard protein hydrolysate:sugar (1:3) diet on fecundity and survival of adult *C. capitata*

Diets	Mean eggs/female/day		% survival	
	Exclude eggs on food	Include eggs on food	Females	Males
Protein hydrolysate:sugar 1:3	24.21 ± 2.07a	24.21 ± 2.07ab	97.50 ± 0.70a	95.60 ± 1.20a
C. capitata #2 agar diet	7.27 ± 2.35c	26.36 ± 1.17a	82.78 ± 2.86ab	90.28 ± 1.80a
C. capitata #2 liquid diet	16.75 ± 2.25ab	16.75 ± 2.25bc	86.67 ± 2.67ab	87.78 ± 2.22a

Means within a column followed by different letters were statistically different ($\alpha = 0.005$, ANOVA test).

Effect of Nutrients on Adult *C. capitata*. Tsiropoulos (1980a) demonstrated that deletion of all vitamins from a chemically defined diet developed for *B. oleae* resulted in a significant decrease in fecundity and fertility while survival remained normal. Kaur and Srivastava (1991) also reported that diet without B-vitamins, folic acid, or biotin reduced oviposition and egg viability. Unlike *B. oleae* and *B. cucurbitae*, adult *C. capitata* fed on *C. capitata* #2 diet without 14 vitamins did not produce significantly fewer eggs or have lower fertility than those fed complete *C. capitata* #2 diet, and survival of both sexes was not affected. Adult *C. capitata* fed on *C. capitata* #2 diet, either without the 10 essential amino acids or the eight nonessential amino acids, significantly reduced egg production in comparison to those fed the complete *C. capitata* #2 diet (Table 2). These results are similar to those described by Ferro and Zucoloto (1990). However, individual deletion of cholesterol, inositol, or choline from the *C. capitata* #2 diet did not affect egg production, whereas the combined deletions of cholesterol, inositol, and choline significantly reduced egg production. This reduction could be attributed to the importance of cholesterol in vitellogenin synthesis (Dwived and Shukla 1982). Survival of adults fed on the above diets was not significantly affected for either males or females. These results suggest that amino acids (when grouped as either essential and nonessential) or cholesterol, choline, and inositol (when combined as a group) are essential for fecundity while vitamins are not indispensable nutrients.

Sugar content in the *C. capitata* #2 diet did not affect egg production but significantly influenced male survival (Table 2). This result supports previous studies with *B. oleae* (Tsiropoulos 1983). Survival of sugar only-fed males and females was significantly lower than those from either the standard diet-fed or *C. capitata* #2 (containing 17.6% sugar) defined diet-fed flies but having similar mortalities to those of 4.4% sugar defined diet.

Results from the liquid *C. capitata* #2 diet were similar to those from the standard diet, but were inferior to the agar formulation (Table 3). This is probably related to the manner in which the food was dispensed. The liquid diet was dispensed with dental wicks that sometimes may block the delivery of certain trace amounts of essential nutrients, whereas the agar diet was blended homogenously with agar. It appears that the *C. capitata* #2 agar diet is the best diet tested because it supported maximum reproductive potential. However, the purpose of this study was to develop a complete chemically defined diet that would be similar to or better than the currently used protein hydrolysate/sugar (1:3) diet. We propose to use this defined diet as a base diet to study nutritional requirements of *C. capitata* adults to further understand the importance of various nutrients in adult fruit fly diets.

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